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The Flavor and Fragrance High Production Volume Consortia

The Alicyclic Aldehyde Consortium

Test Plan for HMPCC

3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (HMPCC) **CAS No. 31906-04-4**

FFHPVC Alicyclic Aldehyde Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:

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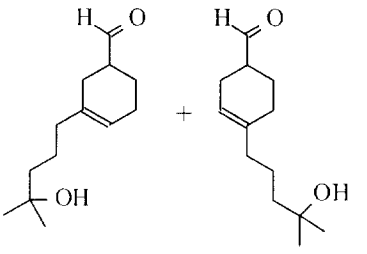
Test Plan for HMPCC

1 IDENTITY OF SUBSTANCE



3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde

CAS No. 31906-04-4

Synonyms:

HMPCC
3-Cyclohexen-1-carboxaldehyde, 4-(4-hydroxy-4-methylpentyl)-
Hydroxyisohexyl 3-cyclohexen carboxaldehyde
4-(4-Hydroxy-4-methylpentyl)cyclohex-3-enecarbaldehyde
Kovanol

2 CHEMICAL ANALYSIS

2.1 INTRODUCTION

In October of 1999, members of the U.S. flavor and fragrance industries as well as other manufacturers that produce source materials used in flavors and fragrances formed consortia of companies in order to participate in the Chemical Right-to-Know Program. Members of these consortia are committed to assuring the human and environmental safety of substances used in flavor and fragrance products. The consortia are organized as the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The Alicyclic Aldehyde Consortium, as a member of FFHPVC, serves as an industry consortium to coordinate testing activities for alicyclic aldehyde substances under the Chemical Right-to-Know Program. Two (2) companies are current members of the Alicyclic Aldehyde Consortium. The Consortium and its member companies are committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and where needed, conducting additional testing. The test plan, category analysis and robust summaries presented represent the first phase of the Consortium's commitment to the Chemical Right-to-Know Program.

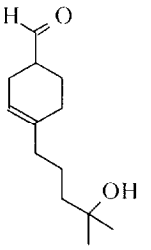
2.2 BACKGROUND INFORMATION

This category analysis and test plan provides data for 3- and 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (from herein referred to as HMPCC) and its structural relatives, hydroxycitronellal, hydroxycitronellol, and perilla aldehyde. HMPCC has not been reported to occur naturally. It is a colorless viscous liquid with a sweet aroma reminiscent of lily of the valley [Bauer and Garbe, 1985]. The functional aroma is similar to that of its naturally occurring counterpart, 7-hydoxycitronellal (*i.e.*, 7-hydroxy-3,7-dimethyloctanal). Therefore, it is not unexpected that HMPCC and 7-hydroxycitronellal contain the same functional groups (*i.e.*, an aldehyde and dimethyl substituted tertiary alcohol) distributed at either end of a carbon chain 8 to 10 carbons in length. Due to the method of preparation (see Section 2.4) HMPCC exists as

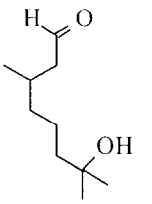
a 70:30 mixture of the 4- and 3-(4-hydroxy-4-methylpentenyl)-3-cyclohexenecarboxyaldehyde isomers. This mixture is the predominant product of commerce. At low concentrations, the mixture has excellent fixative properties, particularly in soap, cosmetics, and perfumes.

2.3 Structural Classification

The chemical structure of HMPCC and 7-hydroxycitronellal feature an aldehyde function and a dimethyl substituted tertiary alcohol located at either end of a carbon chain 8 to 10 carbons in length. In the case of HMPCC, the aldehyde is bonded to a cyclohexene ring that is substituted at the 4 position with a 4-hydroxy-4-methylpentyl substituent. The aldehyde group is separated from the dimethyl substituted tertiary alcohol moiety by seven carbons. In 7-hydroxycitronellal, the aldehyde is bonded to an eight carbon chain containing a 7-hydroxy-7-methyl substituent. In this case, the aldehyde group is separated from the dimethyl substituted tertiary alcohol by five carbons (see structure below).



HMPCC



7-Hydroxycitronellal

2.4 Industrial Production

HMPCC is synthesized by a Diels-Alder reaction in which the double bond of acrolein (2-propenal) adds to the 1- and 4-positions of myrcenol (6-methylene-2-methyl-7-octen-2-ol). At elevated temperature in the absence of a catalyst, the Diels-Alder orientation of addition of the acrolein unit to the diene of myrcenol yields a 30:70 mixture of 3- and 4-(4-hydroxy-4-methylpentyl)-3-cyclohexenecarboxaldehyde. The mixture is the typical product of commerce.

2.5 ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

The structurally related substances 7-hydroxy-3,7-dimethyloctanal (*i.e.*, 7-hydroxycitronellal) and 4-isopropenyl-1-cyclohexenecarboxyaldehyde (*i.e.*, perilla aldehyde) participate in the same pathways of metabolism in rabbits, dogs, rats and humans. The predominant metabolic pathways involve either reduction of the aldehyde to yield the corresponding alcohol or oxidation of the aldehyde to yield the corresponding carboxylic acid. In both cases the metabolites are excreted as glucuronic acid conjugates predominantly in the urine.

Male rabbits (6) were administered an aqueous solution (20 ml) containing Tween 80 (0.02 g/100 ml) and 250-333 mg/kg bw of 7-hydroxycitronellal by stomach tube followed by 20 ml of water. Greater than 35% of the original dose is excreted in the urine as acidic and neutral metabolites within 72 hours [Ishida *et al.*, 1989]. The two principal urinary metabolites of 7-hydroxycitronellal are 7-hydroxycitronellic acid and 7-hydroxycitronellol isolated in a 5:2 ratio, respectively.

Another aldehyde structurally related to HMPCC, 4-isopropenyl-1-cyclohexenecarboxyaldehyde, commonly recognized as perilla aldehyde, was also investigated in the same study. Six male rabbits were given single oral doses of 666 to 800 mg/kg bw of 4-isopropenyl-1-cyclohexenecarboxyaldehyde by gavage followed by 20 ml of water. Approximately 50% of the original dose is isolated within 72 hours. The principal acidic urinary metabolites accounting for approximately 40% of the dose include corresponding carboxylic acid, 4-isopropenyl-1-cyclohexenecarboxylic acid and its aromatized derivative 4-isopropylbenzoic acid. The principal neutral metabolites accounting for approximately 10% of the dose include the corresponding alcohol, 4-isopropenyl-1-cyclohexenecarbinol and its dihydro isomer.

The group of substances, 4-isopropenyl-1-cyclohexenecarboxyaldehyde (perilla aldehyde), 4-isopropenyl-1-cyclohexenecarbinol (perillyl alcohol), and 4-isopropenyl-1-

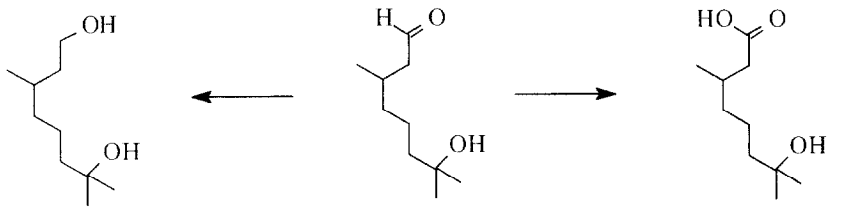
cyclohexenecarboxylic acid (perillic acid) have been the subject of extensive metabolic and toxicologic investigation in rats, dogs, and humans. The substances are currently being investigated as potential anti-carcinogenic agents for therapeutic use in humans. Patients with various advanced malignancies were treated with oral doses of 2,400 mg/m^2/dose of 4-isopropenyl-1-cyclohexenecarbinol for four weeks. Peak plasma levels for the two main metabolites occur at 1.5 hours (4-isopropenyl-1-cyclohexenecarboxylic acid) and 3.5 hours (4-isopropenylcyclohexanecarboxylic acid) post-ingestion. The parent alcohol is not detected in the plasma. The major acid metabolites as well as a small amount of 4-isopropenyl-1-cyclohexenecarbinol (less than 1%) are detected in the urine [Ripple *et al.*, 1998].

An *in vivo* study conducted in male Wistar rats, confirmed that the oxidation of 4-isopropenyl-1-cyclohexenecarbinol to 4-isopropenyl-1-cyclohexenecarboxylic acid involved 4-isopropenyl-1-cyclohexenecarboxaldehyde as an intermediate. Groups of rats were intravenously administered the perillyl alcohol, perilla aldehyde, or perillic acid at a dose of 80 micromoles/kg bw (approximately 12.2, 12.0, or 13.3 mg/kg bw, respectively). Urine and bile were collected for two consecutive hours post administration. In all cases, the glucuronic acid conjugate of 4-isopropenyl-1-cyclohexenecarboxylic acid was the predominant metabolite detected in the urine and bile. Based on the results, the authors concluded that within two hours, approximately 56% of the original dose is oxidized to 4-isopropenyl-1-cyclohexenecarboxaldehyde, followed by the conversion to 4-isopropenyl-1-cyclohexenecarboxylic acid, and eventually excretion as a glucuronic acid conjugate [Boon *et al.*, 2000].

Female Wistar-Furth rats fed a diet of 2% 4-isopropenyl-1-cyclohexenecarbinol for a period of 3, 5, or 10 weeks, show 4-isopropenyl-1-cyclohexenecarboxylic acid and 4-isopropenylcyclohexanecarboxylic acid as major plasma metabolites. Unchanged 4-isopropenyl-1-cyclohexenecarbinol is not detected. The same plasma metabolites are identified four hours after female Wistar-Furth rats are administered a single dose of 1,000 mg/kg 4-isopropenyl-1-cyclohexenecarbinol *via* gavage. No trace of 4-isopropenyl-1-cyclohexenecarbinol is found at any point, including 15 minutes post gavage. These results indicate that 4-isopropenyl-1-cyclohexenecarbinol is rapidly metabolized to 4-isopropenyl-1-

cyclohexenecarboxaldehyde and then to 4-isopropenyl-1-cyclohexenecarboxylic acid. [Haag and Gould, 1994]. Two beagle dogs (male and female) administered 250 mg/kg bw of 4-isopropenyl-1-cyclohexenecarbinol by gavage exhibit peak plasma levels of oxidized metabolites of 4-isopropenyl-1-cyclohexenecarbinol (*e.g.* 4-isopropenyl-1-cyclohexenecarboxylic acid and 4-isopropenylcyclohexanecarboxylic acid) at one and five hours post administration, respectively. Analysis of blood specimens collected before dosing and at 19 points ranging from 10 minutes to 48 hours after dosing, indicate the presence of the oxidized metabolites 10 minutes post administration. The parent substance, 4-isopropenyl-1-cyclohexenecarbinol, is undetectable in the plasma. [Phillips *et al.*, 1995].

Figure 1 - Metabolism of (±)-7-Hydroxy-3,7-dimethyloctanal in Rabbits



7-hydroxy-3,7-dimethyloctanol 7-hydroxy-3,7-dimethyloctanal 7-hydroxy-3,7-dimethyloctanoic acid

2.6 SUMMARY FOR CHEMICAL ANALYSIS

Based on pharmacokinetic and metabolic studies in rabbit, rats, dogs, and humans with 7-hydroxycitronellal and perilla aldehyde derivatives, it is anticipated that HMPCC will be rapidly absorbed *via* the oral route of exposure and primarily metabolized to the corresponding carboxylic acid and, to a lesser extent, the corresponding alcohol. Both metabolites are excreted primarily in the urine.

3 TEST PLAN

3.1 CHEMICAL AND PHYSICAL PROPERTIES

3.1.1 Melting Point

Being a mixture of 3- and 4-(4-hydroxy-4-methylpentenyl)-3-cyclohexene carboxyaldehyde, HMPCC is a viscous liquid at ambient temperature. The calculated melting point for a single isomer of HMPCC according to the MPBPWIN program is between 65.64 and 89.01 °C depending on the method used with a mean of 77.32 °C [MPBPVP EPI Suite, 2000]. Given that the commercial product is a mixture of the 3- and 4-(4-hydroxy-4-methylpentenyl)-3-cyclohexenecarboxyaldehyde, the determination of a melting point for the either the 3- or 4-isomer is not relevant. Based on these calculated values, the melting point of a single isomer of HMPCC is estimated to be 77.32 °C.

3.1.2 Boiling Point

The boiling point of HMPCC has been reported to be 280 °C [FMA, unpublished report] and 120-122 °C at 1.0 mm Hg [Bauer and Garbe, 1985]. The calculated boiling point for HMPCC according to the MPBPWIN program is 307 °C [MPBPVP EPI Suite, 2000]. This value is expected to be higher than that of 7-hydroxycitronellal, a structurally related substance also possessing the same two functional groups located at either end of the carbon skeleton, but containing three additional carbons. Therefore, the boiling point of HMPCC is anticipated to be higher than the 241 °C boiling point [FMA, unpublished report] (85-87 °C at 1.0 mm Hg [Bauer and Garbe, 1985]) reported for 7-hydroxycitronellal. Based on the consistency of the measured and calculated values, the boiling point of HMPCC is recognized to be 280 °C or 120-122 °C at a reduced pressure of 1.0 mm Hg.

3.1.3 Vapor Pressure

The calculated vapor pressure of HMPCC and 7-hydroxycitronellal has been reported to be 0.001 mm Hg at 20 °C [FMA, unpublished report]. The calculated vapor pressure for HMPCC according to the MPBPWIN program was 0.0000273 mm Hg at 25 °C [MPBPVP EPI Suite, 2000]. Although the vapor pressure of HMPCC determined from boiling point data is reported to be less than 0.001 mm Hg at 20 °C, it is recommended that the vapor pressure should be determined by a standardized methodology.

3.1.4 n-Octanol/Water Partition Coefficients

Log KOW was calculated resulting in values of 3.32 [KOWWIN EPI Suite, 2000] and 2.03 [Interactive Analysis LogP and LogW Predictor]. Based on calculated and measured values for 7-hydroxycitronellal, it is likely that the measured log KOW for HMPCC is less than the calculated value. The log KOW of 7-hydroxycitronellal was calculated to be 2.11 [KOWWIN EPI Suite, 2000] while the experimentally determined value is 1.5 [Procter and Gamble, 1996]. These conclusions should be validated by measurement of log KOW for HMPCC.

3.1.5 Water Solubility

The calculated water solubility was estimated to be 1,045 mg/L [Interactive Analysis LogP and LogW Predictor] and 184.6 mg/L at 25 °C [WSKOWIN EPI Suite, 2000]. Based on the limited data set, it is necessary to perform a water solubility test for HMPCC.

3.1.6 New Testing Required

- Vapor pressure for HMPCC according to an OECD Guideline protocol.
- Log KOW for HMPCC according to an OECD Guideline protocol.
- Water solubility for HMPCC according to an OECD Guideline protocol

3.2 ENVIRONMENTAL FATE AND PATHWAYS

3.2.1 Photodegradation

The calculated half-life value for HMPCC has been reported to be 1.009 hours [AOPWIN EPI Suite, 2000]. The short half-life is consistent with the presence of a reactive hydroxyl OH and an aldehyde function.

3.2.2 Stability In Water

HMPCC is expected to be stable in aqueous solution given that it contains an unreactive tertiary alcohol group and aldehyde that is not readily oxidizable in water.

3.2.3 Biodegradation

In a 20-day OECD closed bottle test, HMPCC showed measurable bio-oxidation (BOD/COD x100=10%) after 20 days incubation with activated sludge. Because HMPCC showed limited solubility in the test medium, it was directly injected into the reaction vessel. According to the authors, the test was intended to screen substances of potential biodegradation. [Waggy and Blessing, 1986]. The MITI linear and non-linear model predictions indicate that HMPCC should be readily biodegraded [BIOWIN EPI Suite, 2000].

The structurally related substance, 7-hydroxycitronellal at an initial dose of 52.5 mg DOC/l is completely biodegraded (99.8%) by day 19 in Method F biodegradability study in the Blue Book [Stickley, 1990]. In a more recent biodegradability test using a modified Sturm procedure in an OECD 301B Guideline, 7-hydroxycitronellal was 93.7% biodegraded after 28 days. According to the authors, hydroxycitronellal is classified as readily and ultimately biodegradable [King, 1994].

Although it is anticipated that HMPCC like 7-hydroxycitronellal will be readily and ultimately biodegradable, it is recommended that HMPCC be subjected to a biodegradability study according to a standard OECD Guideline protocol.

3.2.4 Fugacity

Transport and distribution in the environment were modeled using Level III Fugacity-based Environmental Equilibrium Partitioning Model [Mackay, 1991, 1996a, 1996b] through the EPA EPI Suite 2000 program. The input parameters used were molecular weight, melting point (77.3°C), vapor pressure (0.001 m Hg), and boiling point (280°C).

The model predicts that HMPCC is distributed mainly to the soil (74.5%), but also is distributed to water (24.7%) and, to some extent, air (0.015%) and sediment (0.81%).

3.2.5 New Testing Required

- Biodegradation study for HMPCC according to an OECD Guideline protocol.

3.3 ECOTOXICITY

3.3.1 Acute Toxicity to Fish

In a semi-static test with guppies, the structural relative 3-cyclohexene-1-carboxaldehyde exhibited a 14-day LC50 of 10.2 micromoles/L or 21.4 mg/L [Deneer *et al.*, 1988]. The calculated LC50 values for HMPCC are on the same order of magnitude. The calculated 96-hour LC50 for HMPCC was reported to be 6.8 mg/L (aldehydes) and the 14-day LC50 was reported to be 20. mg/L [ECOSAR EPI Suite, 2000].

Given the current database of information, it will be necessary to perform an acute fish toxicity test for HMPCC.

3.3.2 Acute Toxicity to Aquatic Invertebrates

Measured and calculated aquatic invertebrate LC50 values are available for HMPCC. Based on a protocol in EPA Methods for Toxicity Tests with Aquatic Organisms (40GTW23), the 48-hour LC50 for HMPCC in *Daphnia magna* was determined to be 76 mg/L [Waggy and Blessing, 1986]. In *Daphnia magna,* a calculated 48-hour LC50 of 1.773 mg/L was determined [ECOSAR EPI Suite, 2000].

Given the current database of information, it will not be necessary to perform additional acute aquatic invertebrate toxicity tests.

3.3.3 Acute Toxicity to Aquatic Plants

A calculated 96-hour EC50 of 7.091 mg/L was reported for green algae [ECOSAR EPI Suite, 2000].

No experimental data on the aquatic plant toxicity of HMPCC or a structurally related substance are available. Therefore, it is recommended that HMPCC be subjected to an acute toxicity test in green algae.

3.3.4 New Testing Required

- Acute toxicity test of HMPCC in fish according to OECD Guideline 203 protocol.
- Acute toxicity test of HMPCC in algae according to OECD Guideline 201 protocol.

3.4 HUMAN HEALTH TOXICITY

3.4.1 Acute Toxicity

The acute toxicity of HMPCC was reported to be low, with oral LC50s of 3,000 to greater than 5,000 mg/kg bw in rats and dermal LC50s of greater than 5000 mg/kg bw in rabbits [Opdyke, 1977; Mallory *et al.*, 1982; Myers *et al.*, 1987]. Similar results were reported with hydroxycitronellal [Opdyke, 1973].

Exposure to statistically generated saturated vapor of HMPCC for 6 hours, resulted in no deaths, no toxicity or no remarkable gross pathological lesions in exposed male or female rats [Myers *et al.*, 1987].

Groups of rats were exposed in a dynamic system to up to 558 ppm of 4,4-dimethyl-3-cyclohexenecarboxaldehyde vapor for 4 hours and observed for 14 days [Union Carbide, 1987]. Similarly groups of rats were exposed to up to 402 ppm 4,4-dimethyl-3-cyclohexenecarboxaldehyde vapor for 1 hour in a static system and observed for 14 days. Signs exhibited included lacrimation, peri-oral wetness and respiratory difficulties on day of exposure. No clinical signs or macroscopic lesions were reported post exposure. Some deaths occurred on days 1 or 2 post exposure at 558 ppm; however the authors considered the deaths to be related to exposure to acrolein, a reaction precursor and contaminant, since the 1-hour rat LC50 of acrolein is 26 ppm. Therefore, no mortalities were attributed 4,4-dimethyl-3-cyclohexenecarboxaldehyde exposure.

Given the results of oral, dermal, and inhalation studies, no additional acute toxicity tests in mammals are recommended.

3.4.2 *In vitro* and *In vivo* Genotoxicity

3.4.2.1 In vitro

HMPCC did not increase the number of revertants in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 or in *Escherichia coli* strain WP2 *uvr*A when tested with or without metabolic activation at concentrations up to 5,000 micrograms/plate [Wagner and Klug, 1999]. Another structural relative of HMPCC, 2,4-dimethyl-3-cyclohexene-1-carboxaldehyde, also did not increase the number of revertants in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 when tested with or without metabolic activation [Vergnes and Morabit, 1995].

In Chinese hamster ovary cells, HMPCC (at concentrations tested up to 900 micrograms/ml) did not induce an increase in the number of chromosome aberrations in the presence or absence of S9 at any test concentration compared to control solutions [Gudi and Schadly, 2000].

3.4.2.2 In vivo

Groups of mice were intraperitoneally injected with up to 900 mg/kg bw of HMPCC in corn oil [Gudi and Krsmanovic, 2000]. At 24 or 48 hours, mice were killed, femurs were exposed, and bone marrow was removed. The number of micronucleated normochromatic erythrocytes was counted and the proportion of polychromatic erythrocytes to total erythrocytes was determined. HMPCC does not induce micronucleated polychromatic erythrocytes in the mouse bone marrow assay.

When administered to mice by intraperitoneal injection, 7-hydroxycitronellol (up to 1,204 mg/kg bw) and 7-ydroxycitronellal (up to 861 mg/kg bw) did not induce an increase in the incidence of micronuclei in mouse bone marrow [Wild *et al.*, 1983].

In *Drosophila*, 7-hydroxycitronellal (37 mM) or 7-hydroxycitronellol (10 mM) did not increase the number of sex-linked recessive lethal mutations as compared to controls [Wild *et al.*, 1983].

3.4.2.3 Conclusions

The genotoxicity database on HMPCC and 7-hydroxycitronellal shows no mutagenic potential in the Ames assay. In a mammalian assay, there was no evidence of an increase in the incidence of chromosomal aberrations in the presence or absence of S9. In whole animals, the genotoxicity results for HMPCC, 7-hydroxycitronellol, and 7-hydroxycitronellal showed no evidence of an induction of bone marrow micronuclei in mice. In *Drosophila*, 7-hydroxycitronellol or 7-hydroxycitronellal did not induce an increase in the number of sex-linked recessive lethal mutations. Based on these results no additional genotoxicity tests are recommended.

3.4.3 Repeat Dose Toxicity

Repeat-dose inhalation and oral studies have been conducted for structural relatives of HMPCC, including 7-hydroxycitronellal, dimethyl-3-cyclohexenecarboxaldehyde, and perilla aldehyde (4-isopropenyl-1-cyclohexenecarboxyaldehyde) derivatives.

3.4.3.1 Subacute Studies

Rats were exposed to 0, 50, 125 or 250 ppm of 4,4-dimethyl-3-cyclohexenecarboxaldehyde vapor, 6 hours/day for 9 exposures [Norris and Kintigh, 1994]. Additional rats were assigned to the control and high concentration groups for inclusion in a 4-week recovery period. No overt signs of toxicity were reported. There was an increased incidence of higher values for bilirubin, urobilinogen and amorphous phosphates in all treated males on day 11 and an increased incidence of bilirubin and urobilinogen in females at the highest concentration on day 12. Other reported effects included initial decreased body weight gain, increased water consumption, increased serum urea nitrogen values, exposure-related increase in renal tubular

immunohistochemical staining for *alpha*-2micro-globulin in males, increased relative liver (mid- and high doses) and kidney weights (high dose) in males, swollen periocular tissue (mid- and high doses), periocular encrustation (high dose), alopecia (high dose) corneal lesions (high-dose), and increased urine osmolalities in males on day 11 (mid- and high doses). After the recovery period, females exposed to the highest concentration had decreased total erythrocytes, hemoglobin, hematocrit, and mean corpuscular hemoglobin concentration values, showed increased segmented neutrophils and decreased monocytes, and showed slightly increased protein in the urine. The authors considered that dimethyl-3-cyclohexenecarboxaldehyde appears to be an ocular and respiratory irritant at vapor concentrations of 125 ppm and higher. There were no observable adverse effects reported at 50 ppm.

3.4.3.1 Subchronic Studies

Groups of female rats or Syrian golden hamsters were exposed to 211 micrograms of 7-hydroxycitronellal/cubic meters as part of a complex fragrance mixture (50 mg/cubic meters for 4 hours/day, 5 days/week for 13 weeks [Fukayama *et al.*, 1999]. Twelve animals per test group were exposed in a whole body inhalation experiment. Aerodynamic mean diameter of particle size was 0.5 um in rats and 1.4 um in hamsters. Animals were sacrificed 1 to 2 days following exposure. Hematological examination at week 13, involved measurement of white blood cell count, mean corpuscular volume, hemoglobin concentration, and hematocrit. Clinical chemistry examinations were performed at weeks 6 or 7 and week 13. At necropsy, gross pathological examination was performed on 24 organs and tissues including the uterus, testes and ovaries. Histopathological examination was performed on the trachea, lungs, adrenals, brain, esophagus, heart, kidneys, liver pancreas, spleen, sternum, testes, uterus and bone marrow taken from the femur. No toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters were reported and no gross pathological or histopathological findings were observed in either species.

Numerous animal studies have shown high dietary levels of perilla aldehyde (4-isopropenyl-1-cyclohexenecarboxyaldehyde), its corresponding alcohol and acid may be protective against known animal carcinogens. The perilla aldehyde metabolite, 4-isopropenyl-1-cyclohexenecarbinol (discussed above in Section 2.5), has been shown to inhibit the growth of pancreatic, mammary, and liver tumors in animals. It has been studied in animals as a chemotherapeutic agent for neuroblastoma, prostate and colon cancer, and has possible chemotherapeutic applications for skin and lung cancer (Belanger, 1998; Crowell, 1997; Stark *et al.*, 1995; Burke *et al.*, 1997; Mills *et al.*, 1995; Ren and Gould, 1998; Haag and Gould, 1994; Reddy *et al.*, 1997: no robust summaries included).

In a 90-day study, dose levels 40, 120, or 400 mg/kg bw per day of 4-isopropenyl-1-cyclohexenecarbinol was given by gavage to groups of rats and dogs of unspecified number and strain [National Cancer Institute, 1996]. Clinical signs observed with increasing dose included hyper-excitement and a clear mouth discharge. The animals were provided food and drinking water *ad libitum* and monitored for survival, behavior and changes in hematology. In rats, no agent related deaths, abnormal hematology, clinical chemistry or gross lesions were reported at dose levels up to and including 400 mg/kg bw per day. Histopathological examination of major organs and tissues including the ovaries and gonads failed to reveal any alterations that could be associated with administration of the test substance [National Cancer Institute, 1996].

3.4.3.2 Chronic Studies

Two groups of male and female rats were fed 7-hydroxycitronellal at dietary concentrations of 0.1% (10 rats/sex) or 0.5% (20 rats/sex), respectively, for 2 years. These levels correspond to calculated average daily intakes of 50 or 250 mg 7-hydroxycitronellal/kg bw. Control animals were fed a basal diet. Animals were observed for appearance and behavior and body weights were determined regularly during the study. At the end of the study, rats were necropsied and microscopic examinations were performed on the liver, heart, pancreas, adrenals, spleen, brain, and gross lesions. The number of animals that survived the 2-year duration of the study was 5of 10 and 31 of 40, respectively. Low survival was attributed to the occurrence of spontaneous

diseases that occurred at the same rate in both test and control animals. Administration of 7-hydroxycitronellal at dose levels up to 250 mg/kg bw/d resulted in no evidence of systemic toxicty [Bar and Griepentrog, 1967].

3.4.4 Reproductive Toxicity

No reproductive toxicity study on HMPCC or a related compound was available.

3.4.5 Teratogenicity/Developmental Toxicity

No teratogenicity or developmental toxicity study on HMPCC or a related substance was available.

3.4.6 New Testing Required

- A reproductive/developmental screening assay of HMPCC according to OECD 421 Guideline protocol.

3.5 TEST PLAN TABLE

Chemical	Physical-Chemical Properties				
	Melting Point	Boiling Point	Vapor Pressure	Partition Coefficient	Water Solubility
CAS No. 31906-04-4 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde	Calc	A, Calc	Test, Calc	Test, Calc	Test, Calc

Chemical	Environmental Fate and Pathways			
	Photodegradation	Stability in Water	Biodegradation	Fugacity
CAS No. 31906-04-4 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde	Calc	NA	Test, A, R, Calc	Calc

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	Acute Toxicity to Aquatic Invertebrates	Acute Toxicity to Aquatic Plants
CAS No. 31906-04-4 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde	Test, Calc	A, Calc	Test, Calc

Chemical	Human Health Data					
	Acute Toxicity	Genetic Toxicity *In Vitro*	Genetic Toxicity *In Vivo*	Repeat Dose Toxicity	Repro-ductive Toxicity	Develop-mental Toxicity
CAS No. 31906-04-4 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde	A	A	A	R	Test	Test

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties
O	Other

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